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DOI:
10.1038/mp.2011.125

Document Version
Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):
Hollingworth, P., Sweet, R., Sims, R., Harold, D., Russo, G., Abraham, R., Stretton, A., Jones, N., Gerrish, A., Chapman, J., Ivanov, D., Moskvina, V., Lovestone, S., Priotsi, P., Lupton, M., Brayne, C., Gill, M., Lawlor, B., Lynch, A., ... Natl Inst Aging Late-Onset Alzheim (2012). Genome-wide association study of Alzheimer's disease with psychotic symptoms. *Molecular Psychiatry*, *17*(12), 1316-1327. [N/A]. https://doi.org/10.1038/mp.2011.125

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Download date: 05. May. 2023

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Mol Psychiatry. Author manuscript; available in PMC 2013 June 01.

Published in final edited form as:
Mol Psychiatry. 2012 December ; 17(12): 1316–1327. doi:10.1038/mp.2011.125.

Genome-wide Association Study of Alzheimer's disease with Psychotic Symptoms

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Abstract

Psychotic symptoms occur in approximately 40% of subjects with Alzheimer's disease (AD) and are associated with more rapid cognitive decline and increased functional deficits. They show heritability up to 61% and have been proposed as a marker for a disease subtype suitable for gene mapping efforts. We undertook a combined analysis of three genome-wide association studies (GWAS) to identify loci that a) increase susceptibility to an AD and subsequent psychotic symptoms; or b) modify risk of psychotic symptoms in the presence of neurodegeneration caused by AD. 1299 AD cases with psychosis (AD+P), 735 AD cases without psychosis (AD-P) and 5659 controls were drawn from GERAD1, the NIA-LOAD family study and the University of Pittsburgh ADRC GWAS. Unobserved genotypes were imputed to provide data on > 1.8 million SNPs. Analyses in each dataset were completed comparing a) AD+P to AD-P cases, and b) AD+P cases with controls (GERAD1, ADRC only). Aside from the APOE locus, the strongest evidence for association was observed in an intergenic region on chromosome 4 (rs753129; 'AD+PvAD-P' $P=2.85 \times 10^{-7}$; 'AD+PvControls' $P=1.11 \times 10^{-4}$). SNPs upstream of *SLC2A9* (rs6834555, $P=3.0\times10^{-7}$) and within *VSNL1* (rs4038131, $P=5.9\times10^{-7}$) showed strongest evidence for association with AD+P when compared to controls. These findings warrant further investigation in larger, appropriately powered samples in which the presence of psychotic symptoms in AD has been well characterised.

Keywords

Alzheimer's disease; psychosis; behavioural symptoms; genome-wide association study; genetic

Introduction

Alzheimer's disease (AD), the most common form of dementia, is highly heritable (heritability of up to 76%) but genetically complex[1]. Neuropathologically, the disease is characterised by extracellular senile plaques containing β-amyloid (Aβ) and intracellular neurofibrillary tangles containing hyperphosphorylated tau protein[1]. Prior to 2009, four genes had been definitively implicated in its aetiology. Mutations of the amyloid precursor protein (*APP*) gene and the presenilin 1 and *2* genes (*PSEN1, PSEN2*) cause rare, Mendelian forms of the disease usually with an early-onset. Until recently, only apolipoprotein E (*APOE*) had been established unequivocally as a susceptibility gene for the common late-onset form of AD[1].

In 2009 we published a genome-wide association study (GWAS) of AD using the Genetic and Environmental Risk in AD Consortium 1 (GERAD1) sample[2], which identified two genome-wide significant susceptibility loci: *CLU* ($P=8.5\times10^{-10}$) and *PICALM* ($P=1.3\times10^{-9}$). A second independent AD GWAS performed using the European Alzheimer's Disease Initiative sample (EADI) showed genome-wide significant evidence for association with *CLU* ($P=7.5\times10^{-9}$) and *CR1* ($P=3.7\times10^{-9}$), and support for *PICALM* ($P=3\times10^{-3}$). The associations in *CLU*, *PICALM* and *CR1* have been replicated in several independent datasets[3-6] and shown relationships with neurodegenerative processes underlying disease[7]. In addition, Seshadri and colleagues reported genome-wide significant association for *BIN1* ($P=1.6\times10^{-11}$) when combining GERAD1 and EADI data with data from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE)[8].

More recently, members of this consortium have reported findings from an extended study by GERAD (GERAD+), which included 19870 AD cases and 39846 controls and identified genome-wide evidence for association at the *ABCA7* locus ($P=5.0\times10^{-21}$) and the *MS4A* gene cluster ($P=1.2\times10^{-16}$)[9]. The American Alzheimer's Disease Genetic Consortium

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(ADGC) also reported genome-wide significant evidence at the *MS4A* gene cluster, further support for *ABCA7* and suggestive evidence for association with SNPs at the *CD33*, *CD2AP*, *ARID5B* and *EPHA1* loci[10]. When combining data from ADGC and GERAD+ SNPs at *CD33* (P=1.6×10^{-9}), *CD2AP* (P=8.6×10^{-9}) and *EPHA1* (P=6.0×10^{-10}) also exceed criteria for genome-wide significant association with AD.

Despite the success of first generation genome-wide association studies, a substantial proportion of the heritability for complex diseases remains unexplained[11]. It is becoming increasingly apparent that within diagnostic categories, such as bipolar disorder and schizophrenia, extensive aetiological and genetic heterogeneity operates[12]. As a result, there is increasing emphasis on the use of sub-phenotypes to elucidate genotype-phenotype relationships. Psychotic symptoms have been proposed as a marker for a subtype of AD suitable for gene mapping efforts[13]. Psychotic symptoms are significantly more common in AD than the general population, affecting around 40% of cases[14]. They are associated with decreased quality of life for caregivers and patients[15], more rapid cognitive[16] and functional decline[17] and premature institutionalization[15]. We have previously demonstrated familial aggregation of psychosis in AD in three independent cohorts, and estimate that the heritability of AD+P is around 61%[18-21]. Candidate genes for other psychiatric disorders in non-demented populations have been investigated in relation to AD+P, implicating alterations in the serotonergic, dopaminergic, catecholaminergic and noradrenaline neurotranmission systems[22]. However, these findings have not been consistently replicated.

Two possible genetic models of AD+P are a heterogeneity model in which alleles predispose to the development of AD pathology and subsequent psychosis and a disease modifier model in which they modify risk of psychotic symptoms in the presence of neurodegeneration caused by AD [22]. The current study, the first GWAS of AD+P, evaluated these two models by contrasting AD+P with a) healthy controls (heterogeneity model) and b) with AD-P subjects (disease modifier model), utilising existing GWAS data from three independent samples: GERAD1[2], the National Institute on Aging's Late Onset Alzheimer's Disease Family Study (NIA-LOAD)[21, 23] and the University of Pittsburgh Alzheimer Disease Research Center (ADRC)[24].

Methods

Sample Ascertainment

This study included samples from the GERAD1[2], the NIA-LOAD study [23] and the University of Pittsburgh ADRC[24] GWAS datasets.

The GERAD1[1] sample comprised 543 AD+P cases, 454 AD-P Cases and 4701 controls. All AD cases and 955 controls were genotyped at the Sanger Institute on the Illumina 610-quad chip. These data were combined with 3,746 unscreened population controls from the 1958 British Birth Cohort (1958BC; http://www.b58cgene.sgul.ac.uk) and UK Blood Service control group genotyped using either the Illumina HumanHap550 BeadChip (n=2683) or the Illumina HumanOmni1-Quad (n=1063). All AD cases met criteria for either probable (NINCDS-ADRDA[25], DSM-IV) or definite (CERAD[26]) AD. All elderly controls were screened for dementia using the MMSE or ADAS-cog or were determined to be free from dementia at neuropathological examination. All individuals in the GERAD sample were of Caucasian ancestry.

Recruitment for the NIA-LOAD cohort has been described previously[21, 27]. In brief, 18 AD centers throughout the US, each of which had received approval by their institutional review board, participated. The recruitment criteria included a family with multiple members affected with late-onset AD that could provide clinical information and a biological sample

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for DNA extraction. The current report drew from among all such subjects those who had been previously genotyped by the Center for Inherited Disease Research using the Illumina 610-quad chip (Illumina, San Diego, CA, USA). The minimum genotype completion rate for any sample released by CIDR was 98.3%. Blind duplicate reproducibility was 99.99 % based on 118 paired samples[23]. A total of 260 AD+P and 125 AD-P subjects from 264 families were analyzed. Those in the NIA sample were predominantly of Caucasian ancestry (96.1%), but also included 12 (3.1%) individuals classified as African American and 2 (0.5%) individuals of Native American descent.

University of Pittsburgh ADRC subjects were recruited from the University of Pittsburgh AD Research Center, where all subjects undergo a standard assessment[28] and received diagnoses of possible or probable AD with age of onset ≥ 60. Autopsy confirmation rates in similarly diagnosed subjects at this center are >90%[29]. A total of 496 AD+P, 156 AD-P subjects and 958 age-matched screened controls of Caucasian ancestry were included. All AD cases met criteria for either probable (NINCDS-ADRDA[25], DSM-IV) or definite (CERAD[26]) AD. Control subjects included volunteers at the University of Pittsburgh ADRC who underwent the same assessment and were found to be cognitively normal and also populuation-based controls who were found to be cognitively normal using a previously described neuropsychological screening battery[30], which included the MMSE and several additional tests tapping cognitive domains known to be affected in dementia. Genotyping for Pittsburgh subjects was performed using the Illumina Omni1-Quad chip (containing probes for 1.13 million SNPs).

Characterisation of Psychotic Symptoms

The Neuropsychiatric Inventory (NPI)[31] was used to assess behavioural symptoms in all GERAD cases. The NPI is an established and commonly used informant-based rating scale that evaluates 12 common behavioural symptoms in AD[32]. The severity and frequency of each symptom are rated from 0-3 and 0-4, respectively, and were scored to reflect the worst episode of each symptom over the lifetime of the illness. Frequency and severity scores are multiplied to give an overall domain score for each symptom ranging from 0-12. 42% of the GERAD sample were assessed annually for ≥2 assessments. Where multiple NPI ratings were available the highest delusions and hallucinations domains scores were used. AD+P was defined as either the presence of delusions *and* hallucinations, or where only one symptom was present a delusions domain score ≥4 or a hallucinations domain score ≥2. A more stringent cut-off for delusions was adopted to avoid phenocopy due to transient confabulations[13]. AD cases with delusion and hallucination domain scores of 0 were coded as ‘Alzheimer’s with no psychosis’ (AD-P). Individuals with intermediate scores were excluded from analysis.

ADRC and NIA-LOAD subjects were rated for psychotic symptoms on the informant-based CERAD behavioural rating scale (CBRS)[33]. 69% and 35% of the ADRC and NIA-LOAD sample were assessed on more than one occasion. A delusion was defined as a false belief based on incorrect inference about external reality, resistant to persuasion or contrary evidence, and not attributable to social or cultural mores. Hallucinations were defined as sensory perceptions for which there was no basis in reality. Discrete hypnagogic and hypnopompic hallucinations, as well as symptoms occurring only during episodes of delirium, were not rated. The CBRS was administered at initial and annual visits and in some subjects between annual visits by telephone[21, 34]. AD+P was considered present when any of the CBRS items #33 - #45 were rated as occurring ≥3 times in the past month at any visit. Individuals with scores of 0 on the same CBRS items at all visits were classified as AD-P. Inter-rater reliability of the psychosis assessments used, including telephone assessments, has been previously described for the ADRC[22] and NIA-LOAD[21] Cohorts.

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Psychotic symptoms typically emerge in the moderate stages of AD[21, 35], therefore those categorised as AD-P who were in the mild stages of disease at their last assessment (Global Deterioration Scale[36] score<4, Clinical Dementia Rating[37] score <1 or mini-mental state examination score[38] >=20) were considered to be at substantial risk of going on to develop delusional or hallucinatory behaviour. These individuals were therefore excluded from the analysis. As such a total of 219, 5 and 32 individuals were excluded from the GERAD, NIA-LOAD and ADRC samples respectively. Subjects with a known history of mood disorders, bipolar disease, unipolar disease, or an anxiety disorder were also excluded from all analyses.

Quality Control—Quality control (QC) of the GERAD1 sample has been described in detail elsewhere[2]. Briefly, individuals were retained if they had a missing genotype rates < 0.01, with mean autosomal heterozygosity between 0.33 and 0.34, and mean X-chromosome heterozygosity either <0.02 for males, or between 0.25 and 0.40 for females. Genetic outliers and those showing evidence of relatedness (IBD estimate ≥ 0.125) or non-European ancestry based on genotype data were also excluded. Following QC 543 AD+P cases, 454 AD-P cases and 4701 controls were retained. Markers were excluded if they had a minor allele frequency (MAF) < 0.01 or a Hardy-Weinberg $P \leq 1 \times 10^{-5}$. SNPs with a MAF≥0.05 were excluded if they had a genotype missing rate of >0.03; for SNPs with a MAF between 0.01 and 0.05, a more stringent genotype missing rate threshold of 0.01 was employed.

Individuals in the NIA-LOAD sample were retained if they had a missing genotype rate < 0.05. 260 AD+P and 125 AD-P subjects falling in 264 families passed QC. Markers were excluded if they had a minor allele frequency (MAF) < 0.05 or if they had a genotype missing rate of >0.02. No modifications were made for Hardy-Weinberg equilibrium given the small sample size. 516,835 SNPs passed QC.

QC and analysis of the ADRC sample has been described in detail elsewhere (Kamboh et al. manuscript in preparation). Briefly, individuals were retained if they had a missing genotype rate <0.02, with mean X-chromosome heterozygosity either <0.02 for males or between 0.25 and 0.4 for females. Genetic outliers and those showing evidence of relatedness (IBD estimate ≥ 0.4) or non-European ancestry based on genotype data were excluded. Following QC, 496 AD+P cases, 156 AD-P cases and 958 unaffected controls were retained. Markers were excluded if they had a minor allele frequency (MAF) <0.01 or a Hardy-Weinberg $P \leq 1 \times 10^{-6}$ in controls. SNPs were also excluded if they had a genotype missing rate of >0.02. Markers were examined to determine if the rate of missing data was found to depend on case/control status or the genotyping batch. No additional exclusions were needed based on these analyses.

Following quality control this study comprised a total of 1299 cases with AD+P, 735 individuals characterised as AD-P and 5659 controls.

Imputation—As there is only moderate overlap between the Illumina 550/610 arrays (used in the GERAD1 and NIA-LOAD GWAS) and the Illumina Omni1-Quad array (used in the ADRC GWAS) unobserved genotypes were imputed. The GERAD genotype data was imputed with MACH v.1.0, using haplotypes released from initial low coverage sequencing of 112 European ancestry samples in the 1000 genomes project (ftp://ftp.sanger.ac.uk/pub/1000genomes/REL-0908/LowCov/) as a reference sample. The phasing step included 200 individuals to calculate the specific maps based upon the sample recombination rates. Imputation generated data for >8.2 million SNPs. On the investigation of several QQ – plots from an explorative association analysis, an a-posteriori filter was applied to exclude SNPs with MAF<0.01 or r^2<0.3. The ADRC data was also imputed with MACH v.1.0, using haplotypes from the HapMap v3 data release as a reference sample. Imputation generated

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data for >3 million SNPs that were subsequently filtered to exclude SNPs with $r^2<0.3$. Imputed genotypes were not available for the NIA-LOAD dataset

Association analyses

GERAD1: Separate analyses were completed to compare AD+P with unaffected controls and AD-P cases. Following QC 4659431 and 4479280 SNPs were tested in the 'AD +PvControl' and 'AD+PvAD-P' analyses, respectively. Analyses were completed using logistic regression assuming an additive model. The first four principal components extracted from an EIGENSTRAT[39] analysis were included as covariates.

ADRC: Separate analyses were performed to compare AD+P with unaffected controls and AD-P cases in the ADRC sample. Following imputation and QC, a total of 2543888 SNPs were tested in each analysis. Analyses were performed using logistic regression assuming an additive model in PLINK[40]. Age (age at exam for unaffected controls or age-at-diagnosis for AD cases), sex and the first four principal components extracted from a multi-dimensional scaling analysis (also implemented in PLINK) were included as covariates in the analyses.

NIA-LOAD: The NIA-LOAD sample included AD cases sampled from multiplex families. As some of the subjects were related, the contrast of AD+P versus AD-P was performed using Generalized Estimating Equations or GEE methods. For simplicity we assumed all relative pairs were related as full-siblings, a conservative assumption, and accounted for their estimated covariance in tests contrasting AD+P versus AD-P allele frequencies. To account for differences in genetic ancestry, cases and controls were matched based on genetic distances calculated from the 8 significant dimensions of ancestry as determined by SpectralGem[41].

Meta-analyses—An inverse variance weighted fixed effects meta-analysis was used to test for association with AD+P in the GERAD1, NIA-LOAD and ADRC datasets. Separate meta-analyses were completed for 'AD+PvControls' (including summary statistics from GERAD1 and ADRC) and 'AD+PvAD-P' (including summary statistics from GERAD1, NIA-LOAD and ADRC). Cochran's Q-test was performed and I^2 calculated to assess heterogeneity. SNPs present, and passing all QC filters, in 2 or more studies were included. All analyses were completed using METAL (http://www.sph.umich.edu/csg/abecasis/metal/)[42].

Results

A total of 1299 AD+P cases, 735 AD-P cases and 5659 controls were included in the meta-analysis. Clinical characteristics of the sample can found in Table 1. The final meta-analysis of the 'AD+PvControl' and 'AD+PvAD-P' datasets included 1847262 and 1882172 SNPS, respectively. The genomic control inflation factors (λ)[43] were 1.002 and 1.004 respectively, suggesting little evidence for residual stratification. Table 2 shows loci associated ($P<1.0\times10^{-5}$) with AD+P when compared either controls or AD-P cases. Summary statistics for SNPs with P-values $\leq 1\times10^{-4}$ can be found for 'AD+Pvcontrols' and 'AD+PvAD-P analyses in Supplementary Tables 1 and 2, respectively. Figure 1 includes manhatten plots of the 'AD+PvControls' and 'AD+PvAD-P' GWAS.

Excluding the *APOE* region, the most significant SNP (rs753129) had a P-value of 2.85×10^{-7} (OR= 0.66) and was associated with AD+P when compared to AD-P cases. rs753129 is in a intergenic region on chromosome 4. The same SNP showed some evidence for association in analysis of 'AD+PvControls', OR= 0.81, $P=1.11\times10^{-4}$. The most significant SNP outside the APOE locus in the 'AD+PvControl' analysis was rs6834555, which sits just

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upstream of the solute carrier family 2 (facilitated glucose transporter), member 9 (*SLC2A9*) gene (OR=1.39, P=3.0×10^{-7}). This SNP was not associated with AD+P when compared to AD-P (OR=1.13, P=0.18). The most significant intragenic SNP in the 'AD+PvControl' analysis was rs4038131, an intronic SNP in the visinin-like 1 (*VSNL1*) gene (OR: 0.65, P=5.9×10^{-7}). This SNP was also associated with AD+P when compared to AD-P cases, (OR= 0.72, P=1.84×10^{-2}).

No evidence of association was observed at the APOE locus when analyzing 'AD+PvAD-P' (all P>0.235). Six SNPs in close proximity to APOE showed genome-wide significant (GWS) associations when comparing AD+P cases with controls. All of these markers showed similar patterns of effect and more compelling evidence of association in the primary analysis of the GERAD+ Stage 1 analysis, which did not condition on presence or absence of psychotic symptoms (P-values ranging from 3.5 × 10^{-39} – 1.6 ×10^{-215})[44]. Furthermore none of these SNPs showed evidence of association with AD+P when compared to AD-P (P>0.330), indicating that the *APOE* locus is associated with AD, unconditional of psychosis status. Results for these SNPs at the APOE locus can be found in Supplementary Table 3.

Recent GWAS studies of AD have identified compelling evidence for nine novel risk loci for AD: *CLU, PICALM, CR1, BIN1, ABCA7, MS4A, CD2AP, CD33 and EPHA1*[2, 8-10, 45]. In this study, comprising a subset of the GERAD[9], NIA-LOAD[10] and University of Pittsburgh ADRC[24] samples included in the studies which identified these loci, we noted association (P<0.05) with *BIN1* (rs744373, P=2.6×1^{-5}, OR=0.80), *EPHA1* (rs11767557, P=0.002, OR=1.22), *CLU* (rs11136000, P=0.005, OR 0.86), MS4A (rs670139, P = 0.028, OR=1.12) and *PICALM* (rs3851179, P=7.2×10^{-5}, OR=0.81) when comparing AD+P cases with controls. The pattern of effect was consistent with that observed in the primary analysis of the GERAD+ dataset and none of these SNPs showed evidence of association when comparing AD+P and AD-P cases, suggesting that these loci are associated with AD, irrespective of presence or absence of psychotic symptoms (see Supplementary Table 4).

Finally, we investigated whether putative risk loci for schizophrenia (SZ) or bipolar disorder (BP), both of which feature prominent psychotic symptoms, were associated with AD+P when compared to AD-P or Controls (Table 3). We tested those SNPs that have shown genome-wide significant evidence for association (P≤ 5.0 × 10^{-8}) in GWAS of SZ or BP. Eleven SNPs were chosen, six of which were identified by GWAS of schizophrenia[46-48] five by GWAS of BP[49-51]. Although individually GWA SNPs showed only limited association with AD+PvAD-P, as a group there was a trend towards association, combined P=0.109 (using an unweighted Z-transformation[52], excluding rs1938526 and rs13194053 as they are in strong LD with rs10994336 and rs6932590 respectively) We also identified 5 additional loci without genome-wide significant evidence for association in SZ or BP, but implicated by meta-analysis as listed in the top 10 SZGene loci on 1st June 2011 (http://www.szgene.org/). These SNPs showed no evidence of association with AD+PvAD-P individually, or as a group (Combined P=0.90), see Table 3.

Discussion

A substantial body of evidence indicates that the presence of psychotic symptoms in AD identifies a subgroup of subjects who undergo more rapid cognitive and functional decline[22]. The distinctive nature of the AD+P phenotype, in conjunction with evidence of the familial aggregation and heritability of psychosis in AD, has led to the hypothesis that AD+P may have a genetic architecture which diverges from that of AD-P[13]. There are two plausible genetic models of AD+P. First, a heterogeneity model in which alleles predispose to the development of AD pathology and subsequent psychosis and secondly, a disease

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modifier model in which alleles increase the risk of psychosis conditioned on the presence of AD[22]. The current study evaluated these two models by contrasting AD+P with healthy controls and with AD-P subjects.

Although we did not observe novel genome-wide significant evidence of association with AD+P when compared to healthy controls or AD-P cases, several additional considerations apply. Despite testing for association in one of the largest cohorts of AD+P and AD-P subjects studied to date, the power of the present study to detect loci of the magnitude commonly observed in complex traits is limited. For example, for common variants (MAF= 0.3) the current study has 0.02 and 0.06 power to detect disease susceptibility loci with OR of 1.2 in the 'AD+PvAD-P' and 'AD+PvControl' analyses respectively, and power is even less for lower frequency alleles that confer this effect size. It is therefore possible that many SNPs in the current study, while failing to reach this stringent level of statistical significance, could reflect true disease loci with resulting implications for the biology of AD +P. For example, the most significant intragenic SNP in the 'AD+P v Control' analysis was rs4038131, an intronic SNP in the visinin-like 1 (*VSNL1*) gene. *VSNL1* utilizes a calcium-myristoyl switch mechanism that upon stimulation by intracellular calcium causes *VSNL1* to translocate to cell membranes, including the transGolgi apparatus, where it may influence a number of cell signalling pathways[53]. *VSNL1* concentrations are elevated in the cerebrospinal fluid (CSF) of individuals with AD, where they correlate with CSF tau concentrations and with the degree of cognitive impairment[54]. Whether *VSNL1* expression in cerebral cortex of AD+P subjects is reduced in comparison to AD-P subjects is not known, but *VSNL1* mRNA and protein expression are reduced in cerebral cortex of subjects with schizophrenia[55, 56]. Another SNP that showed suggestive evidence of association when comparing AD+P cases with controls was rs6834555, which is located just 5′ of the urate transporter, *SLC2A9*. Variation in *SLC2A9* has been shown to influence serum urate concentrations across populations[57, 58]. Of interest, reduced serum urate concentration has been identified in AD[59] and in schizophrenia[60, 61]. Serum and cerebrospinal fluid urate have also been shown to be positively correlated[59]. How altered urate concentration may relate to the brain pathologies observed in schizophrenia and AD is not known, though the protective antioxidant effects of urate have been suggested as one potential mechanism[59-61]. Relatively less evidence currently exists to suggest that the SNPs identified in the 'AD+PvAD-P' analysis may be tied to the neurobiology of AD or psychosis. One exception may be serine/ threonine kinase 11 (*STK11*). An unusually large scale STK11 deletion has been described in one case in which Peutz-Jeghers syndrome, mental retardation, and schizophrenia co-occurred[62] and reduced copy numbers of *STK11* have been reported in affected siblings with schizophrenia[63].

In contrast to the above genes, we found little evidence to suggest that previously identified AD risk genes may contribute selectively to the risk of psychosis within AD. Several of these loci showed association when contrasting AD+P cases with controls, however the effect sizes were comparable to those obtained when comparing AD cases unselected for psychotic symptoms with controls. Furthermore, none of these loci showed association in the 'AD+PvAD-P' analysis. This conclusion should be tempered by awareness of the limited power in our 'AD+P v AD-P' comparison.

We previously identified 22 studies, comprising more than 5,200 subjects with AD, that examined the association of the APOE ϵ4 allele with AD+P, with nine reporting significant associations[22]. Though not supportive of a true assocation of *APOE* with AD+P, such a pattern could result from false negatives due to variation between studies in the in subject populations, sample sizes, definitions of AD+P and analytic approaches, or due to limited power to detect small, but real effects (e.g. O.R. 1.1-1.2). A recent examination of the largest sample to date of AD subjects uniformly characterized for psychosis (N=2317) found that

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neither *APOE* ε4 carrier status nor allele number was associated with psychosis[64]. Alternatively, the variable pattern of association might reflect causal association with genetic variation in linkage disequilibrium with the *APOE* ε4 allele. The current study, which found no association with AD+P of SNPs throughout the chromosome 19 region containing *APOE*, tested in more than 2000 AD cases characterized for psychosis, does not support this alternative. Similarly, when examined in samples which largely overlap with the current ADRC cohort, there was no evidence of association of AD+P with a poly-T repeat length variation in *TOMM40* which is in linkage disequilibrium with *APOE*[65]. Taken as a whole, these data do not support a role of *APOE* in risk for psychosis within AD subjects, a finding consistent with a current meta-analysis of 26 studies which finds no association of *APOE* ε4 alleles with risk for schizophrenia (www.szgene.org).

These analyses are largely hypothesis generating. It is now important to follow up these findings, either through replication or meta analysis of other AD GWAS datasets where psychotic symptoms have been well characterised. As such we will make the complete genome-wide meta-analysis results for 'AD+PvAD-P' and 'AD+PvControls' available to researchers upon application. It also notable that this study was limited to mainly those of Caucasian origin. Future studies may seek to investigate AD+P within other ethnic groups. It is interesting to note some overlap between schizophrenia and psychosis in AD was observed. There is increasing evidence that there is genetic overlap between the major psychiatric[66, 67] and nonpsychiatric[68] disorders. The largest family study of bipolar disorder and schizophrenia has recently been published. They showed that there are increased risks of both schizophrenia and bipolar disorder to first-degree relatives of probands with either disorder, which was largely due to additive genetic effects[66]. Furthermore, a recent study provides strong evidence that schizophrenia is largely polygenic, involving many common SNPs that are substantially shared with bipolar disorder[67]. It is plausible that this overlap will extend to other disease phenotypes with prominent psychotic symptoms. A vital part of dissecting the large amounts of GWAS data available in psychiatric and neuropsychiatric populations will be working toward more biologically valid classification approaches, which will allow genetic mappings of symptoms domains and dimensions which will undoubtedly lead to greater understanding of clinical phenotypes[69].

It is important to note that there are no established criteria for defining AD+P. We took several approaches in order to minimize the possibility of misclassification of subjects. Because rates of AD+P increase with disease progression, we used all available longitudinal structured ratings of behavioural pathology within subjects to identify AD+P as the occurrence of symptoms at any time point. We excluded individuals with transient mild psychotic symptoms to avoid phenocopies due to misunderstanding of confusional episodes. Similarly, we excluded non-psychotic individuals in the earliest stages of illness, who may have been at risk of expressing psychosis only after their illness progressed. These operationalised criteria for AD+P map closely to those used in our earlier studies that demonstrated familial aggregation of AD+P in AD[18, 21]. Nevertheless, we cannot rule out the possibility that because the extent of longitudinal data varied for individuals, some individuals may have been misclassified with regard to their psychosis status. It is also notable that there were different proportions of individuals in each sample categorised as AD+P and AD-P. This is likely reflects differences in the availability of longitudinal ratings and disease severity, subtle differences in rating instruments and also differences resulting from the underlying populations studies. By performing, analyses in each cohort, controlling for population substructure, and combining results through meta-analyses we limit the effect these differences will have on the results of this study.

This study also included unscreened, population-based controls in the AD+P v Control analysis. Unscreened controls are commonly used in GWAS studies and can substantially

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increase power to detect true genetic associations where disease prevalence is low (K_p<0.2)[70]. It is noteworthy that the GERAD GWAS, which includes a large proportion of unscreened controls, has already produced compelling evidence for new susceptibility genes for AD: CLU, PICALM, BIN1, CR1, ABCA7, MS4A, CD2AP, CD33 & EPHA1. Of which CLU, PICALM, BIN1, CR1, ABCA7 and MS4A have since been replicated in independent samples[3, 5, 7, 71-73]. Despite the pragmatic value of unscreened controls, there are potential limitations to their use. Though the population prevalence of AD+P is low, that largely reflects its age dependency, thus a high proportion of individuals among unscreened controls may carry genetic risk for AD+P and would be anticipated to manifest this syndrome if they were evaluated through the age of risk. As such, contrasts between AD+P and unscreened controls may fail to detect some meaningful genetic associations. Also, due to age differences between AD subjects and unscreened controls some SNPs associated with AD+P in the 'ADPvControl' comparison may represent confounds due to a true association with a survival advantage. These potential limitations are mitigated somewhat in the current study by the inclusion of screened elderly controls. Similarly, loci with evidence of association in both the 'AD+PvControl' and the 'AD+PvAD-P' (e.g. VSNL1) are less likely to result from this confounding.

In conclusion, this study does not identify any SNPs that meet strict criteria for genome-wide significant association with AD+P, when compared to controls or AD-P cases. However a number of sub threshold associations were observed that a) show patterns of effect that are stronger than those generally observed in AD GWAS, b) are interesting biological and position candidates for AD+P and c) show some overlap with others psychiatric disorders with psychotic features. As such these findings warrant further investigation in larger, appropriately powered samples in which the presence of psychotic symptoms in AD is well characterised.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

GERAD1: We thank the patients and families who took part in this research. The Cardiff University group was supported by the Wellcome Trust, Medical Research Council, Alzheimer's Research Trust and the Welsh Assembly Government. The Alzheimer's Research Trust also supported and funded DNA sample collections at the Institute of Psychiatry, Cambridge University, University of Nottingham and University of Belfast. The University of Belfast group are supported by the Alzheimer's Society, Alzheimer's Research Trust, Ulster Garden Villages, N Ireland R&D Office and the Royal College of Physicians/Dunhill Medical Trust. The Trinity College Dublin sample was supported by the MRC and Mercer's Institute for Research on Ageing. The LASER-AD study was funded by Lundbeck SA. GR is supported by a program grant from the MRC (G0800509). We would also like to thank Advanced Research Computing @ Cardiff (ARCCA) who facilitated data analysis.

NIA-LOAD: Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. Samples used in this study were obtained from the National Cell Repository for Alzheimer's Disease (NCRAD). Jennifer Williamson, Susan LaRusse Eckert and Stephanie Doan (Columbia University), Michele Goodman (Indiana University), and Elise Weamer (University of Pittsburgh) helped coordinate the project across the United States. We would especially like to acknowledge the support and guidance of Creighton H. Phelps, PhD, at the National Institute on Aging.

ADRC: The following investigators and Alzheimer's Disease Centers participated in the Study: *Boston University* Robert Green, Neil Kowall, Lindsay Farrer; *Columbia University* Jennifer Williamson, Vincent Santana; *Duke University* Donald Schmechel, Peter Gaskel; *Indiana University*, Bernardino Ghetti, Martin R. Farlow, Kelly Horner; *Massachusetts General Hospital* John H. Growdon, Deborah Blacker, Rudolph E. Tanzi, Bradley T. Hyman; *Mayo Clinic-Rochester* Bradley Boeve, Karen Kuntz, Lindsay Norgaard, Nathan Larson; *Mayo Clinic-Jacksonville* Dana Kistler, Fracine Parfitt, Jenny Haddow; *Mount Sinai School of Medicine* Jeremy Silverman, Michal Schnaider Beeri, Mary Sano, Joy Wang, Rachel Lally; *Northwestern University* Nancy Johnson, Marcel

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Mesulum, Sandra Weintraub, Eileen Bigio; *Oregon Health and Science University* Jeffery Kaye, Patricia Kramer, Jessica Payne-Murphy ; *Rush University* David Bennett, Holli Jacobs, Jeen-Soo Chang, Danielle Arends; *University of Alabama at Birmingham* Lindy Harrell; *University of California, Los Angeles* George Bartzokis, Jeffery Cummings, Po H Lu, Usha Toland; *University of Kentucky* William Markesbery, Charles Smith, Alise Brickhouse; *University of Pennsylvania* John Trojanowski, Vivianna Van Deerlin, Elisabeth McCarty Wood; *University of Pittsburgh* Oscar L. Lopez, Robert A. Sweet; *University of Southern California* I Helena Chui, Arousiak Varpetian; *University of Texas Southwestern* Ramon Diaz-Arrastia, Roger Rosenberg, Barbara Davis; *University of Washington* Thomas Bird, Malia Rumbaugh, Gerard D. Schellenberg, Murray Raskind; *Washington University at St Louis* Alison Goate, John Morris, Joanne Norton, Denise Levitch, Betsy Grant, Mary Coats.

This study was supported by the following federal grants: U24AG026395 (NIA-LOAD Family Study); U24AG021886 (National Cell Repository for Alzheimer's Disease); R01AG027224, R01AG030653 and P50AG005133 University of Pittsburgh; P30AG10161 Rush University Medical Center; P30AG013846 Boston University; P50AG08702 Columbia University; P30AG028377 Duke University; P30AG010133 Indiana University; P50AG05134 Massachusetts General Hospital; P50AG165574 Mayo Clinic, Rochester and Mayo Clinic, Jacksonville; P01AG05138, P01AG02219, and P50AG05138 Mount Sinai School of Medicine; P30AG13854 Northwestern University Medical School; P30AG008017 Oregon Health and Science University; P50AG016582 University of Alabama at Birmingham; P50AG016579 David Geffen School of Medicine, University of California, Los Angeles; P30AG028383 University of Kentucky, Lexington; P30AG10124 University of Pennsylvania; P50AG05142 University of Southern California; P30AG12300 The University of Texas Southwestern Medical Center; P50AG05136 University of Washington; and P50AG05681 and P01AG03991 Washington University School of Medicine.

Collection and ascertainment of the ADRC subjects was supported by USPHS grants AG027224.

We thank contributors who collected samples used in this study, and we particularly thank the patients and their families, whose help and participation made this work possible.

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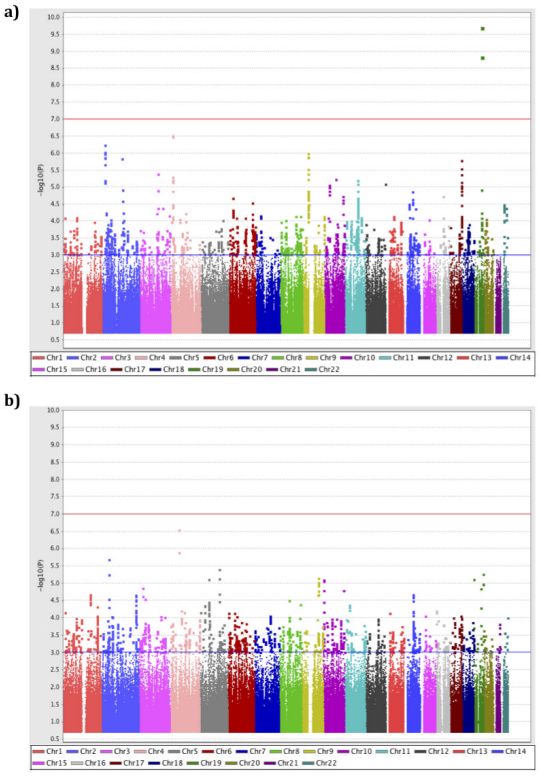

Figure 1.

Scatterplot of chromosomal position (x-axis) against −log 10 GWAS P-value (yaxis). a) AD +P vs Controls, b) AD+P vs AD-P. The threshold for genome-wide significance ($p \leq 9.5\times10^{-8}$) is indicated by the red horizontal line. The *y*-axis scale has been limited to 10 ($P = 1 \times 10^{-10}$), although highly significant association was observed with SNPs in the vicinity of the APOE locus when comparing AD+P cases with controls. Plots produced using Haploview v4.0 (http://www.broad.mit.edu/mpg/haploview/).

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Table 1

Descriptive characteristics for all samples.

	GERAD1	NIA-LOAD	ADRC
Genotyping Platform	**Illumina 610**	**Illumina 610**	**Illumina 1M Omni**
AD+P			
n	543	260	496
% Female	72.9	69.6	66.9
Age at onset, Mean (SD)	75.6 (7.2)	73.4 (7.3)	73.1 (6.2)
Age at Interview/ascertainment, Mean (SD)	80.9 (6.9)	82.5 (7.1)	76.9 (5.8)
AD-P			
n	454	125	156
% Female	67.8	56.0	59.0
Age at onset, Mean (SD)	76.0 (6.8)	74.3 (7.5)	73.4 (6.3)
Age at Interview/ascertainment, Mean (SD)	80.4 (7.0)	81.9 (7.4)	76.4 (5.8)
Screened Controls			
n	955	N/A	958
% Female	61.5	N/A	63.3
Age at Interview/ascertainment, Mean (SD)	75.9 (6.3)	N/A	75.8 (6.5)
Population Controls			
n	3746	N/A	N/A
% Female	50.7	N/A	N/A
Age at Interview/ascertainment, Mean (SD)	48.7 (1.2)	N/A	N/A

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Table 2

Loci associated with AD+P at $P<1\times10^{-5}$ in either the 'AD+PvControl' or 'AD-PvAD-P' analysis.

Analysis	SNP	Chr	MB	MAF	Closest RefSeq gene	Distance (Kb)	GWAS P value	OR	Direction of effect*
AD+PvControl	rs6834555	4	9.7	0.21	SLC2A9	5766	3.06E-07	1.40	+/ /+
AD+PvControl	rs4038131	2	17.6	0.07	VSNL1	Intragenic	5.90E-07	0.65	−/ /−
AD+PvControl	rs4576506	9	31.5	0.06	RP11-291J9.2	39859	1.04E-06	1.66	+/ /+
AD+PvControl	rs10207628	2	127.6	0.19	BIN1	Intragenic	1.46E-06	0.71	−/ /−
AD+PvControl	rs16970672	17	73.5	0.29	AC015804.1	28310	1.67E-06	1.29	+/ /+
AD+PvControl	rs9811423	3	114.3	0.47	RP11-572M11.4	Intragenic	4.18E-06	1.28	+/ /+
AD+PvControl	rs733175	4	9.7	0.18	SLC2A9	Intragenic	4.97E-06	1.37	+/ /+
AD+PvControl	rs4746003	10	71.2	0.25	RP11-242G20.2	426	5.95E-06	1.30	+/ /+
AD+PvControl	rs10792830	11	85.5	0.44	AP003097.1	25461	6.40E-06	1.25	+/ /+
AD+PvControl	rs1464108	12	129.6	0.32	RIMBP2	19600	8.19E-06	1.28	+/ /+
AD+PvControl	rs11006923	10	28.5	0.05	MPP7	Intragenic	8.99E-06	0.63	−/ /−
AD+PvAD-P	rs753129	4	56.4	0.24	AC110611.1	3534	2.85E-07	0.66	−/-/−
AD+PvAD-P	rs2969775	2	47.7	0.37	AC079250.1	32379	2.11E-06	0.68	−/ /−
AD+PvAD-P	rs257016	5	123.2	0.36	AC008541.1	166411	4.06E-06	0.70	−/ /−
AD+PvAD-P	rs6509701	19	58.1	0.30	ZNF320	9	5.41E-06	0.71	−/−/−
AD+PvAD-P	rs16922670	9	105.1	0.14	RP11-341A22.2	Intragenic	7.22E-06	1.63	+/ /+
AD+PvAD-P	rs3764640	19	1.2	0.21	STK11	Intragenic	7.88E-06	0.68	−/−/−
AD+PvAD-P	rs11252926	10	0.6	0.36	DIP2C	Intragenic	8.08E-06	0.72	−/−/−

CHR, Chromosome; MB, position in megabases; MAF, Minor Allele Frequency; OR, odds ratio for the minor allele. Loci listed are those with p<1×10−5 in either the AD+P v Control or 'AD+PvAD-P' meta-analysis.

*Direction of effect is shown for GERAD1, NIA-LOAD and ADRC Studies individually; + indicates the log(OR) of the minor allele is positive (OR>1), − indicates the log(OR) of the minor allele is negative (OR<1), blank if not available. Nearest gene (or microRNA) is listed, with the distance (kb) from the gene (or intragenic) noted. SNPs in LD ($r^2>0.8$) with the top hit at each locus are not shown. Six SNPs at the APOE locus with $P<5\times10^{-8}$ were observed. All of these markers showed similar patterns of effect and more compelling evidence of association in the primary analysis of the GERAD1 dataset, which tested for association AD, irrespective of psychosis status[2]. Furthermore none of these SNPs showed evidence of association with AD+P when compared to AD-P ($P>0.235$), These SNPs can be found in Supplementary 3

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Table 3

Analysis of putative bipolar disorder (BP) and schizophrenia (Sz) loci

Source	SNP	Chr	MB	Locus	AD+P v Control			AD+P v AD-P		
					OR	P	Direction of effect*	OR	P	Direction of effect*
Genome-wide significant SNPS from Schizophrenia or Bipolar GWAS										
SZ GWAS[46], SZGene	rs6932590	6	27.4	*PRSS16*	**0.97**	5.8E-01	−/−	**0.88**	3.3E-01	/−/−
SZ GWAS[46], SZGene	rs3131296	6	32.3	*NOTCH4*	**1.07**	3.3E-01	+/+	**1.16**	1.5E-01	+/+/+
SZ GWAS[46], SZGene	rs9960767	18	51.3	*TCF4*	**0.93**	5.0E-01	−/+	**0.67**	2.7E-02	−/ /−
SZ GWAS[46], SZGene	rs12807809	11	124.1	*NRGN*	**0.99**	8.3E-01	−/+	**0.88**	1.8E-01	−/−/+
SZ/BP GWAS[47], SZGene	rs1344706	2	185.5	*ZNF804A*	**0.99**	7.9E-01	+/−	**0.95**	5.4E-01	+/ /−
BP GWAS[49]	rs1938526	10	62.0	*ANK3*	**0.76**	6.4E-02	/−	**0.72**	1.3E-01	/−/−
BP GWAS[49]	rs10994336	10	61.8	*ANK3*	**1.26**	2.5E-02	+/+	**1.36**	7.1E-02	+/ /+
BP GWAS[50]	rs2251219	3	52.6	*PBRM1*	**1.06**	2.8E-01	+/+	**1.00**	9.5E-01	+/−/+
BP GWAS[49]	rs1006737	12	2.2	*CACNA1C*	**1.01**	8.9E-01	−/+	**1.05**	5.3E-01	+/ /+
BP GWAS[51]	rs1064395	19	19.2	*NCAN*	**1.08**	2.5E-01	+/+	**1.10**	2.9E-01	+/−/+
SZ-GWAS [48]	rs13194053	6	27.3	*MHC*	**0.96**	5.0E-01	−/−	–[b]	–	–
Additional SNPs from SZGene Top 10										
SZGene	rs13211507	6	28.4	*PGBD1*	**1.01**	9.5E-01	/+	**0.94**	7.9E-01	/+/−
SZGene	rs6913660	6	27.2	*HIST1H2BJ*	**1.04**	5.4E-01	+/+	**1.01**	9.5E-01	/−/+
SZGene	rs910694	1	66.6	*PDE4B*	**1.07**	2.0E-01	+/−	**1.08**	3.0E-01	+/ /+
SZGene	rs7192086	16	13.0	*SHISA9*	**0.94**	2.6E-01	−/−	**1.03**	7.4E-01	+/ /−

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Source	SNP	Chr	MB	Locus	AD+P v Control			AD+P v AD-P		
					OR	P	Direction of effect*	OR	P	Direction of effect*
SZGene	rs4646984[a]	11	0.6	*DRD4*	–	–	–	–	–	–

CHR, Chromosome; MB, position in megabases; OR, odds ratio for the minor allele. BP, bipolar disorder, SZ, Schizophrenia

*Direction of effect is shown for GERAD1, NIA-LOAD and ADRC Studies individually; + indicates the log(OR) of the minor allele is positive (OR>1), − indicates the log(OR) of the minor allele is negative (OR<1), blank if not available.

[a]120bp duplication, proxy unavailable in the AD+P datasets,

[b]in LD with rs6913660 (r^2=1, D′=1)